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A tense situation: forcing tumour progression

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Abstract

Cells within tissues are continuously exposed to physical forces including hydrostatic pressure, shear stress, and compression and tension forces. Cells dynamically adapt to force by modifying their behaviour and remodelling their microenvironment. They also sense these forces through mechanoreceptors and respond by exerting reciprocal actomyosin- and cytoskeletal-dependent cell-generated force by a process termed ‘mechanoreciprocity’. Loss of mechanoreciprocity has been shown to promote the progression of disease, including cancer. Moreover, the mechanical properties of a tissue contribute to disease progression, compromise treatment and might also alter cancer risk. Thus, the changing force that cells experience needs to be considered when trying to understand the complex nature of tumorigenesis.

At a glance

- Cells within tissues are continuously exposed to physical forces, including hydrostatic pressure, shear stress and compression and tension forces. The nature of these forces can change in pathologies such as cardiovascular disease and cancer.
- Cells sense force through mechanoreceptors and, regardless of the type of force applied, cells respond by exerting reciprocal actomyosin- and cytoskeleton-dependent cell-generated force by a process termed mechanoreciprocity.
- Mechanoreciprocity maintains tensional homeostasis in the tissue and is necessary for development and tissue-specific differentiation. Its loss promotes disease progression, including liver fibrosis, atherosclerosis and cancer.

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DATABASES

National Cancer Institute Drug Dictionary: <http://www.cancer.gov/drugdictionary/> 5-azacytidine|tamoxifen

UniProtKB: <http://www.uniprot.org> β 5 integrin | β -casein | biglycan | BCAR1 | CCR7 | CREB | decorin | FAK | fibronectin | LOX | lumican | MAL | MECP2 | MMP2 | MMP3 | MMP11 | MMP12 | MMP13 | MYC | NF- κ B | p53 | paxillin | RAP1 | SRF | STAT1 | STAT3 | talin 1 | tenascin | TGF β | TIMP3 | TWIST1 | VEGFA | vinculin

- Cells dynamically adapt to force by modifying their behaviour and remodelling their microenvironment. This adaptation probably involves a combination of epigenetic chromatin remodelling events and direct physical links between the matrix and nucleus that regulate gene expression. These gene-regulatory processes are altered in diseases such as cancer.
- Breast cancer is characterized by changes in cellular rheology and tissue level forces, a stiffening of the tissue and a progressive loss of tensional homeostasis that has been exploited to detect tumours. The mechanical properties of a tissue contribute to disease progression, compromise treatment and might also alter cancer risk.

Force modulates cell fate and directs tissue development and post-natal function. Although we know much about the biochemical pathways that direct cell behaviour, by comparison we know less about how force can regulate cell fate and tissue phenotype. Nevertheless, cells in tissues such as the heart, lung and skeleton encounter nanoscale to macroscale forces that are integral to their function. The nature of these tissue-associated forces can be parallel, such as the shear stress induced by blood flow on a vessel wall, or perpendicular, such as the compressive or tensile stress induced by weight bearing on bone. In fact, all cells, including those incorporated into traditionally mechanically static tissues, such as the breast or the brain, are exposed to isometric force or tension that is generated locally at the nanoscale level by cell–cell or cell–extracellular matrix (ECM) interactions. These nanoscale forces influence cell function through actomyosin contractility and actin dynamics, and it is increasingly clear that force collaborates with biochemical cues to modulate cell and tissue behaviour.

In this Review we summarize the current understanding of tensional homeostasis in tissue development, homeostasis and cancer, and identify important areas for investigation. Defining the role of force on cell and tissue behaviour depends on understanding what contributes to force generation in the tissue, how the cell senses and integrates exogenous mechanical signals within its tissue microenvironment, and thereafter how the cell coordinates its response as part of a multicellular, organized tissue structure within its three-dimensional ECM microenvironment. To focus our Review, we have detailed how force modulates the normal and malignant behaviour of mammary epithelial cells in the context of the breast, illustrating, where pertinent, major concepts with examples from experimental findings.

Forcing form and function

The importance of mechanical force in biological systems is illustrated by exploring its role in normal tissue development and function. The mechanical stress that a cell is subjected to is quantified in Pascals (Pa) and measured as force per unit area, or N per m² (BOX 1). This mechanical stress or force, in turn, is perceived and integrated in the cell at the molecular level through mechanically responsive sensors that interface with biochemical signalling cascades to elicit a specific cellular response through mechano-effectors. For example, force and growth factor receptor signalling can each influence cell growth, survival, motility, differentiation, shape and gene expression by regulating the activity of RhoGTPases that modulate actomyosin contractility and actin dynamics^{1–7}. Similarly, integrin-dependent extracellular-signal regulated kinase (Erk) signalling and focal adhesion assembly are regulated by both growth factor signalling and force from the ECM^{8,9} (BOX 2).

Force and embryogenesis

Force has a fundamental role in directing stem cell fate and in dictating embryonic development^{10–12}. For instance, embryonic stem cells progressively stiffen as cells differentiate¹³, whereas stem cell shape and specification are influenced by Rho-dependent

contractility that is modulated by the mechanical properties of the tissue microenvironment^{3, 5}. Indeed, mesenchymal stem cells undergo lineage selection in response to the elasticity of the matrix substrate. Soft matrices, similar to the brain, direct stem cells into a neurogenic lineage, whereas stiffer matrices, similar to muscle and newly deposited bone, direct them into myogenic and osteogenic lineages³. As development proceeds, tension fields mediated by cell compression that result from normal morphogenic movements also shape the embryo. Indeed, external micropipette-applied force, which mimics these developmental forces, drives nuclear translocation of the transcription factor Armadillo to activate the transcription of twist, which controls the formation of the dorsal–ventral axis in the early *Drosophila melanogaster* embryo¹⁴. Tissue development depends not only on the precisely timed application of force, but also on its correct spatial localization, as in the distinctly patterned tissue domains that specify cell polarity, shape and motility in the trunk and head mesoderm in *Xenopus laevis* embryos^{15, 16}. By contrast, mislocalization of Rho and Rac, which regulate cell contractility, prevents blastula gastrulation^{15, 16}.

Force is essential for normal tissue-specific development, in which it orchestrates tissue organization and function, and regulates cell growth, survival and migration. The lung epithelium, for example, undergoes branching morphogenesis as a result of progressive end bud enlargement and expansion to form the respiratory tree¹⁷. Interestingly, like the branching of the adolescent mammary gland, branch patterning in the lung epithelium is dictated by localized remodelling of the ECM and the corresponding stretching of lung epithelial cells at these locations. Force also regulates the integrity of the final lung ductal tree, which is governed by the cyclic shear stress of fetal breathing movements^{18, 19}. Indeed, compromising Rho-dependent cytoskeletal tension perturbs basement membrane thickness, disrupts terminal bud formation and compromises lung epithelial duct organization²⁰.

Adult tissue homeostasis and the ECM

A balance of forces is required to maintain adult tissue homeostasis. Skeletal health depends on mechanical loading, such that exercise increases the proteoglycan content of articular cartilage whereas reduced mobility leads to loss of proteoglycan content and exacerbates arthritis-associated joint degeneration^{21, 22}. Force also facilitates bone matrix deposition to accommodate skeletal loading such that immobilization of the organism, unilateral lower limb suspension or microgravity leads to loss of bone mineral density, which in turn compromises bone strength^{23, 24}. Similarly, vascular function is largely determined by fluid shear stress^{25, 26}. The shear stress induced by blood flow permits artery maturation by directing endothelial cells and their filamentous cytoskeletal networks to elongate and align with the direction of flow²⁷.

It is becoming increasingly apparent that each tissue has a characteristic ‘stiffness phenotype’ (FIG. 1) and that each cellular component within a tissue has a unique rheology and a stiffness optimum that can change over the course of development (as in lung branching morphogenesis), in response to function (as during mammary gland lactation) or in pathological situations (as in atherosclerotic plaque formation or in tumours)^{28, 29}. Furthermore, the physical properties of the ECM and cellular rheology can profoundly influence cellular behaviours as diverse as differentiation, tissue organization and cell migration^{6, 30–32}.

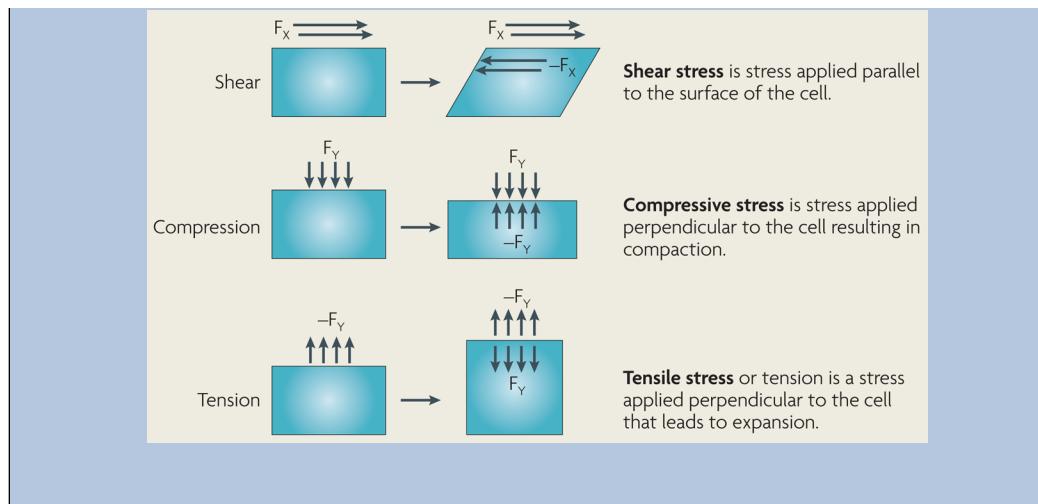
A cell within a tissue is subjected to isometric force through dynamic interactions with the ECM and its neighbouring cells, and these forces exert profound effects on cellular behaviour. For example, endothelial cells form branched capillary-like vessels when cultured within compliant gels, but form vessels with larger lumens in more rigid matrices. Compliance-dependent cell behaviour has also been observed in neural, muscle and mesenchymal cell populations. Therefore, ECM stiffness is an isometric force that exerts its effects gradually and

chronically on cell behaviour, predominantly at the nanoscale level. An increase in ECM protein concentration, increased matrix crosslinking or parallel reorientation of matrix fibrils within a stromal matrix can stiffen a tissue locally to alter cell growth or direct cell migration, albeit to differing degrees. This phenomenon permits fine-tuning of cellular function within a heterogenous tissue.

Interstitial collagens are major contributors to tissue materials properties. Collagens undergo a myriad of post-translational modifications, including matrix metalloproteinase (MMP)-dependent cleavage, glycosylation and crosslinking, that modify their tensile strength and viscoelasticity. During extracellular processing, collagen propeptides are cleaved by specific endoproteinases. Thereafter, enzymes such as the lysyl oxidases (LOX) and the lysyl hydroxylases catalyse covalent intermolecular crosslinks between collagens and with elastin. LOX-mediated crosslinking increases insoluble matrix deposition, tissue tensile strength and matrix stiffness³³. However, chronically increased LOX activity increases collagen crosslinking and this can stiffen heart muscle to compromise cardiac function³⁴. Importantly, non-enzymatic collagen crosslinking, such as glycation and transglutamination, or increased biglycan and fibro-modulin proteoglycan levels also stiffen the matrix³⁵. The excessive deposition of proteoglycans in injured lungs contributes to fibrosis by stiffening the parenchyma³⁶, whereas inappropriate glycation-mediated crosslinking compromises wound healing and cardiac function in diabetic patients in whom glycation is increased owing to high blood glucose levels^{37,38}. Therefore, isometric and active forces have crucial roles in tissue behaviour. Force directs the differentiation of stem cells, drives the assembly of differentiated tissues and maintains tissue homeostasis.

Box 1 | Types of forces experienced by a cell

Normal physiological processes expose cells to a variety of mechanical stimuli including hydrostatic pressure, shear, compression and tensile force. The right-hand images in the figure depict the balance of forces once equilibrium is achieved following the application of a mechanical force. Newton's third law states that for every action there is an equal and opposite reaction and, following this law, cells *in vivo* will respond to alterations in the mechanical properties of their surrounding matrix by adjusting their intracellular tension through the cytoskeletal network. Conversely, changes in cell tension will result in alterations in extracellular matrix (ECM) organization, thereby changing the mechanical properties of the ECM. Stress is defined as a normalized load, where the force or load is divided by the cross-sectional area available to support the load, and the units of stress are Newtons per square metre (N/m^2) or Pascals (Pa). The deformation of a material in response to a given load varies with the geometry and the composition of the specimen. Strain is a normalized deformation, in which the change in length is divided by the original length of the specimen, and is a unitless quantity. We and others have previously measured the stiffness or Young's modulus of tissues *in vivo* and reported values in units of Pascals⁶. Soft biological tissues can be described as viscoelastic materials. A viscous fluid resists shear flow and strain linearly with time under stress. An elastic solid undergoes deformation under stress and rapidly returns to its original state. Viscoelastic biological materials exhibit the characteristics of both a viscous fluid and an elastic solid.



Mechanotransduction and mechanoreciprocity

Given that cells are exposed to a myriad of active and isometric forces, it follows that cells must have derived an array of generic and specialized force-sensory mechanisms. Examples of specialized mechanosensors include the primary cilia in the hair cells of the inner ear and calcium-gated ion channels in cardiac muscle^{39,40}. Activation of stretch-activated potassium channels⁴¹, activation and oligomerization of transmembrane integrins, and remodelling of the cytoskeleton in response to shear flow are examples of conserved mechanosensory mechanisms⁴².

The current contention is that all force sensors directly undergo controllable molecular changes in response to force, regardless of their nature. This behaviour is illustrated by the sequential unfolding of p130Cas (also known as BCAR1) and conformational changes in the integrin-associated molecule talin 1 in response to force^{43,44}. Elegant experiments demonstrated that direct application of a piconewton force can stimulate the mechanical extension of p130Cas, revealing a domain that is a substrate of Src family kinases^{45,46}. Phosphorylation by Src family kinases subsequently activates the small GTPase RAP1 and initiates a sequence of events that propagates integrin signalling^{45,47}. Force-induced conformational changes in talin 1 also reveal a binding site for vinculin, and force can modify extracellular fibronectin to alter integrin adhesion⁴⁸, suggesting other plausible mechanisms by which force could link the ECM to the inside of the cell (FIG. 2a).

Once mechanical cues have been detected, cells must propagate and amplify the physical cue within the cell and translate the signal into either a transient response or sustained cellular behaviour. Integrins, by virtue of their extracellular interaction with the ECM and intracellular interaction with plaque proteins and the cytoskeleton, are an excellent example of one such ubiquitous mechanotransducer^{49,50} (FIG. 2a). Either exogenous or endogenous force can activate integrins, facilitate their nucleation and clustering, and drive their maturation into focal adhesions^{6,51–55}. Integrin oligomerization in turn facilitates RhoGTPase-dependent actomyosin contractility and cytoskeletal reinforcement⁶. Integrin clustering and cytoskeletal reinforcement lead to the phosphorylation of focal adhesion kinase (FAK) at tyrosine 397 (REF. 56), which stabilizes the focal adhesions through activation of small RhoGTPases and actin remodelling. The assembly of focal adhesions perpetuates downstream signalling through kinases and initiates cytoskeletal remodelling through the nucleation of an assortment of adhesion plaque proteins and signalling molecules, including Ras, Rac and Rho^{57,58}. Ras couples force-dependent integrin signalling to MAPKs including Erk, as has been illustrated in lung epithelial cells in response to mechanical strain⁵⁹ and increased Erk phosphorylation

in endothelial cells in response to cyclic strain⁶⁰. Importantly, however, what has yet to be determined is whether any cellular or extracellular protein whose activated conformation can be enhanced by force constitutes a viable mechanosignalling mechanism and, if so, what then dictates mechanospecificity. Indeed, do mechanohierarchies exist?

Force-dependent activation of signalling cascades allows cells to respond quickly to a dynamic force environment, and the same pathways also lead to sustained changes in cell behaviour. Force-activated Erk cooperates with other kinases, such as Src and FAK, to induce cell proliferation or sustain cell survival⁶¹, as shown for MAPK-dependent growth of keratinocytes in response to mechanical stretch⁶² and the load-dependent survival of osteocytes⁶³. In addition to changes in cell growth and survival, compression stress affects microtubule dynamics⁶⁴ to induce quantifiable changes in cell shape, whereas durotactic gradients of ECM stiffness^{30,65} direct cell motility and the migration of fibroblasts and smooth muscle cells. In response to mechanical loading, fibroblasts synthesize and secrete many ECM proteins, including fibronectin, tenascin and collagen, and direct matrix remodelling through the expression, secretion and activation of MMPs and crosslinking enzymes. These sustained cellular responses to force must be coordinated. One factor implicated in this orchestrated response is transforming growth factor- β (TGF β). Mechanical force initiates post-translational activation of secreted TGF β from a latent complex into the functional ligand⁶⁶. TGF β , in turn, can stimulate the production of matrix proteins and matrix-modifying enzymes such as MMPs and LOX that dramatically alter the characteristics of the extracellular stroma⁶⁷. In extreme cases, chronic activation of TGF β can even induce tissue fibrosis and disease in soft tissues such as the liver and kidney⁶⁸. In this manner, cells can dramatically change the composition, topology and elasticity of their tissue microenvironment and alter their adhesions and cell shape and orientation to tune their behaviour according to the magnitude, direction and nature of applied mechanical stress.

Box 2 | Three-dimensional model systems to study the effect of force

Three-dimensional cell culture models offer a distinct advantage over conventional two-dimensional systems because they recapitulate both the architecture and the phenotypical behaviour of differentiated tissues with reasonable fidelity. Three-dimensional model systems can be used to study force and its effects on cell behaviour in the context of an organized tissue structure *in vitro*. These systems use primary or immortalized cells and natural or synthetic hydrogels (for example, collagen I, reconstituted basement membrane, alginate, agarose, synthetic peptides and polyacrylamide). By various means, protein and polysaccharide gels can be manipulated to modify their mechanical properties. An increase in the total protein concentration of protein gels, such as collagen or fibrin, results in an increased stiffness of the polymerized network. In this case, the elastic modulus has been approximated to be proportional to the square of the protein concentration¹⁸⁵. Free-floating or relaxed gels present a more compliant three-dimensional environment to cells than anchored or stressed gels and are more sensitive to cell force generation¹⁸⁶. Glycation by the addition of reducing sugars such as glucose or ribose results in non-enzymatic crosslinking of collagen fibres that can further stiffen the three-dimensional collagen gels¹⁸⁸. The stiffness of fibrin gels can be increased by the addition of salts at physiological pH or by activation of the plasma transglutaminase factor XIII^{188,189}. Altering the protein concentration to change gel stiffness can introduce additional variables into the model system. The use of polyacrylamide gels allows for precise control of the gel stiffness while maintaining ligand density and chemical content and changing either the bis-acrylamide (a polyacrylamide crosslinking agent) or the acrylamide components of the gel can alter the gel mechanics¹⁹⁰. Pelham and Wang pioneered polyacrylamide gels for cell culture less than 10 years ago and numerous investigators have used this model system with many different cell types to address the question of cell response to ECM force. This technique

has proved exceptionally adaptable, such that gels of varying stiffness can be combined to resemble the mechanical properties of, for example, the alveolar basement membrane and breast stroma^{6,191}.

Push-me-pull-you

Cells are not simply passive force recipients but also respond dynamically to externally applied force or stiff matrices with a proportional reciprocal cell-generated force. This reciprocal force response depends on actomyosin contractility and cytoskeletal remodelling. For instance, inside the cell, adaptor proteins associated with focal adhesions such as talin and α -actinin directly link the cytoplasmic domains of β integrin subunits with actin filaments^{69,70}. Actin stress fibres polymerized at the focal adhesion act like viscoelastic cables and respond to the extracellular mechanical environment with myosin-induced cell contractility^{71,72}. Cells anchor to and pull on ECM fibrils, creating intracellular tension⁷³. This intracellular tension, which can be induced experimentally by local application of mechanical stress to the extracellular domains of integrins, redirects cytoskeletal reorganization and ultimately activates RhoGTPases to generate large traction forces that can be measured using traction force microscopy⁷⁴ (FIG. 2b,c). Such approaches have revealed that cell-generated force or mechanoreciprocality can profoundly influence cell behaviour by enhancing cell spreading, growth, survival and motility^{6,30,65}. Indeed, cellular tension and microrheology are finely tuned to the properties of their surrounding matrix, and the nature and magnitude of applied force they experience within their tissue microenvironment. The magnitude of cellular contractility reflects the cell type and state^{75,76}. For instance, cells on stiff substrates tolerate excision of a single stress fibre by exerting greater myosin-dependent force, whereas the same manipulation in cells grown on a compliant substrate disrupts the cellular force balance and cell shape⁷⁷. We have observed that normal mammary epithelial cells generate greater force and occupy more surface area on a stiff matrix (5,000 Pa) than similar cells interacting with a soft matrix of 140 Pa (FIG. 2b).

So, at the single cell level, cell-generated force can increase adhesion strength, enhance integrin-dependent signalling and drive cytoskeletal remodelling to change cell rheology and shape and modify cell behaviour. In multicellular tissues increased cell contractility can destabilize cell–cell adhesions and promote cell invasion to facilitate wound healing or drive MMP-dependent branching morphogenesis^{78,79}. As well as changing cell shape and behaviour, mechanical forces can also alter gene expression.

Gene expression and force

Changes in microenvironment or cell behaviour that permit the long-term adaptation to exogenous forces or alterations in matrix compliance require a change in gene expression. Integrin expression, for example, is much higher in fibroblasts and epithelial cells that are grown on rigid substrates than those that are grown on compliant gels, and the expression of $\alpha 5$ integrin is induced following sustained exposure to a stiffer matrix^{80,81}. So, how might force regulate gene expression? Many of the signalling networks that are activated in response to force, such as Erk and Jun N-terminal kinase (JNK), activate and induce nuclear translocation of transcription factors such as AP1, p53, signal transducer and activator of transcription 1 (STAT1), STAT3, MyC, CCAAT/enhancer-binding protein (C/EBP), cAMP response element-binding protein (CREB) and nuclear factor- κ B (NF- κ B)^{33,82–85}. Therefore, force probably modifies cell fate by altering the activity of various adhesion and growth factor-dependent transcriptional networks. However, acinar morphogenesis within a compliant reconstituted basement membrane (rBM) or in response to mechanical loading is associated with the repression and induction of hundreds of genes, and we determined that human mammary epithelial cells (HMEC) respond to matrix stiffness by altering the expression of at

least 1,500 genes that span multiple functional categories⁸⁶ (K. C. Tsai *et al.*, unpublished data). Likewise, although we showed that breast tumour progression in the HMT-3522 human breast cancer model is associated with specific genomic alterations, the accompanying gene expression profile differs markedly between those cells grown on either a rigid tissue culture plastic or stiff rBM-conjugated polyacrylamide gels and those within compliant rBM or on soft rBM-conjugated polyacrylamide gels⁸⁷. This suggests that additional gene regulatory mechanisms, possibly linked to chromatin remodelling, must also be regulated by force.

A direct mechanical link from the ECM to nuclear chromatin could dynamically alter gene expression in response to exogenous force¹ through a solid-state signalling mechanism that is governed by the principles of ‘tensegrity’ (tensional integrity). The tensegrity model implies that integrins are linked to the nucleus through the cytoskeleton, that an applied force is transmitted to the DNA through the cytoskeleton by nuclear lamins and nuclear envelope receptor complexes, and that this then modulates gene expression by inducing conformational changes in chromatin either by altering the nature of the protein complexes at the telomeres of chromosomes or by changing the activity of DNA remodelling enzymes^{88–92}. Support for this paradigm has come from studies demonstrating how application of force on the integrin–ECM interface can induce nuclear and chromatin distortion⁹³, that tension can alter DNA wrapping⁹⁴, and that speared chromatin can be excised from the nucleus as a continuum that remains physically linked to the cytoskeleton and adhesion interface⁹⁵. Alternately, epigenetic changes regulate gene expression during embryogenesis and tissue-specific development. Given that force also modulates these processes, it follows that mechanotransduction might influence chromatin remodelling to regulate histone acetylation and methylation. For example, HMEC morphogenesis and differentiation in a compliant rBM but not on a stiff two-dimensional substrate is associated with pronounced chromatin remodelling, changes in histone deacetylase (HDAC) expression and activity, and increased expression of the methyl-CpG-binding protein MECP2 (REFS 96,97) (Tsai *et al.*, unpublished data). In addition, we and others have found that rBM compliance dictates the response of differentiated HMEC acini to the methylation inhibitor 5-azacytidine or the HDAC inhibitor trichostatin A. Only on compliant matrices do these inhibitors induce gene expression to sensitize a mammary epithelium to exogenous growth and death stimuli, coincident with a disruption of morphology and cytoskeletal organization^{96,97} (K. Levental, V.M.W. and N. Zahir, unpublished observations). These results indirectly implicate the properties of matrix materials in the control of cell shape, cytoskeleton morphology and chromatin remodelling.

Several studies have highlighted the interactions between force, Rho signalling, cell shape and histone acetylation^{98,99}. Adhesion-induced changes in HMEC shape are associated with altered actin organization, RhoGTPase activity, actomyosin contractility and modified global patterns of chromatin histone acetylation^{6,100}. Similarly, modifying fibroblast adhesion and changing cell shape alters cytoskeletal organization and shrinks the nucleus and nuclear lamina of cultured cells. These changes in the cytoskeleton and nuclear morphology are associated with impaired polymerase access to chromosomal territories and a concomitant reduction in gene transcription^{91,101–103}. More convincingly, Rho-family GTPases indirectly regulate histone H4 acetylation by shifting the balance of cellular and nuclear pools of F and G actin, which in turn, modifies the association between serum response factor (SRF) and its co-activator MAL (also known as MKL1)^{104–106}. These and other data argue convincingly that mechanical force regulates gene expression to alter cell behaviour either by directly altering the DNA or by modulating the function of chromatin remodelling molecules. The current challenge facing biologists then is to delineate the molecular mechanisms underlying these provocative phenomenological observations.

Changes in mechanical stress and cancer

Loss of tissue homeostasis is a hallmark of disease. Given the pluripotent role of force in tissue function, it is not surprising that multiple pathologies, including cancer, are characterized by compromised tensional homeostasis^{6,68,107}. Indeed, tumours are often detected as a palpable ‘stiffening’ of the tissue, and approaches such as magnetic resonance imaging elastography and sono-elastography have been developed to exploit this observation to enhance cancer detection^{108,109}. More provocatively, altered stromal–epithelial interactions precede and can even contribute to malignant transformation (K. Levant and V.M.W., unpublished observations), and the desmoplastic stroma that is present in many solid tumours is typically significantly stiffer than normal⁶. This raises the interesting possibility that preventing tissue stiffening could impede cancer progression, and that genetically susceptible individuals predisposed to matrix stiffening might be at greater risk for tumours and could benefit from enhanced screening programmes^{37,110,111}. To discuss these ideas further, we focus on the role of mechanical stimuli in the regulation of normal breast development and the implications these have for breast cancer.

The mechanics of mammary morphogenesis and maintenance

Force modulates all stages of breast development and is vital to the proper functioning of the differentiated tissue. Together with hormonal and growth cues, force specifies the architecture of the mature ductal tree and mediates efficient delivery of milk to the young. In mammals the breast is the source of nutrients and passive immunity for the offspring, so abnormalities in tensional homeostasis not only impair the structural organization and health of the tissue, but could also compromise the survival of the species. As such, understanding how force orchestrates the behaviour of such a crucial tissue as the breast should provide insight into how mechanics regulates the behaviour of other seemingly mechanically inert tissues.

The mammary gland comprises an organized ductal tree consisting of a single polarized layer of luminal epithelial cells that interact at their basal surface with a network of contractile myoepithelial cells (FIG. 3). Each intralobular ductal tree terminates in a cluster of alveoli, which comprise the basic structural unit of the breast. It is this basic acini unit that will differentiate to produce milk on exposure to lactogenic hormones¹¹². Surrounding the ducts and alveoli of each lobule is the intralobular stroma, which is a loose connective tissue containing microvasculature, small lymphatic channels, adipocytes, resident fibroblasts and inflammatory cells¹¹³. Adjacent to and encompassing the intralobular stroma and ductal network is the interstitial stroma, which comprises over 80% of the human breast volume^{114,115}. Unlike the loose and cellular intralobular stroma, the connective tissue of the interstitial stroma is dense, less cellular and contains variable proportions of ECM and adipose tissue.

A highly organized ECM supports the tissue and cellular level architecture of the breast. Collagen IV, heparin proteoglycans, perlecan and various laminin isoforms comprise the basement membrane that surrounds the mammary epithelial cell (MEC) bilayer. Together these provide mechanical stress shielding that is crucial for functional integrity of the ductal tree^{116,117}. The intralobular stromal matrix, which surrounds the ductal tree, is secreted primarily by stromal fibroblasts and is composed of structural matrix proteins — including collagens I and III, elastin, proteoglycans, glycosaminoglycans and glycoproteins — that interact to form a large complex network in the extracellular space that is contiguous with the basement membrane¹¹⁸. The organization, concentration and crosslinking of the structural components of the basement membrane and the intralobular matrix contribute to their material properties¹¹⁹. Together, the basement membrane, intralobular matrix and interstitial stroma are a continuum that cooperates to define the form and function of the breast through their ability to act as a physical scaffold, to function as a repository for growth factors and cytokines,

and to provide specific biochemical and tensional cues through specialized cellular receptors. Thus, the ECM can be considered a highway by which MECs are able to communicate with one another and with stromal cells locally and distally through biochemical and mechanical cues.

Forces that operate from the nanoscale to the macroscale facilitate normal functioning of the differentiated breast. The nature and magnitude of these forces reflect the organization, composition, topology and post-translational modification state of the ECM and the organization of the ductal tree. Thus, the matrix surrounding the large ducts is more linear and stiffer, whereas the collagen surrounding the terminal ductal units is more relaxed and the matrix is more compliant (K. Levant and V.M.W., unpublished observations). During lactation, the breast is subjected to compressive stress on the luminal and myoepithelial cells and the basement membrane owing to the accumulation of milk and distension of the ducts, which is facilitated by the highly compliant, relaxed collagen matrix surrounding the differentiated acini. Upon suckling, the luminal epithelial cells encounter inward projecting tensile stress as the myoepithelium contracts in response to oxytocin to force the milk out of the alveolar sacs and into the larger ducts to facilitate efficient feeding of the young. In the absence of the suckling stimulus, lactation ceases and milk accumulates within the acini, slowly exerting an outwardly projecting compressive force of increasing magnitude on the surrounding luminal epithelium and myoepithelium. With prolonged milk stasis and continued gland distension, this compressive force eventually compromises the integrity of the tight junctions between luminal alveolar cells, and the gland undergoes involution accompanied by extensive remodelling of the cellular and extracellular stroma^{120,121}. Importantly, gland remodelling dramatically changes the composition and architecture of the stroma. The remodelled stroma consequently alters the signals and the force encountered by the MECs within the ducts and by so doing sets the stage for a subsequent round of epithelial proliferation and differentiation¹²². For example, primary cultures of murine MECs form polarized mammary acini with an endogenous basement membrane and differentiate in response to lactogenic hormones when embedded within a floating collagen gel. By contrast, these same cells will spread and continue proliferating in response to identical stimuli when interacting with a stiff two-dimensional scaffold or incorporated into a mechanically loaded collagen gel¹²³. Similarly, immortalized MECs fail to express one of the major milk proteins, β -casein, unless they interact with a compliant basement membrane^{123–125}.

The crucial role of matrix compliance in MEC morphogenesis was illustrated by studies using matrices with defined viscoelastic properties. HMECs embedded within collagen–rBM gels or interacting with rBM-crosslinked polyacrylamide gels with matrix compliance comparable to the normal murine mammary gland proliferated until they formed growth-arrested, polarized mammary acinus-like structures with a central lumen and an external endogenous basement membrane. When the matrix is progressively stiffened, cell growth is enhanced, cell–cell junction integrity is compromised and lumen formation is impeded. MECs interacting with the most rigid matrices form continuously growing, non-polarized, disorganized and invasive colonies that lack detectable cell–cell junction proteins and exhibit irregular cell shapes with detectable actin stress fibres. Whereas MECs interacting with the highly compliant matrix form nascent focal contacts, those within the stiff gels assemble mature focal adhesions with active FAK phosphorylated on Tyr397, vinculin and p130Cas (REF. 6). Importantly, when MECs engineered to express a constitutively active V14Rho or a mutant V737N integrin that promotes integrin clustering interact with a compliant basement membrane, they exert higher contractility, assemble focal adhesions and display tissue phenotypes characteristic of MECs interacting with a stiff matrix. Such observations underscore the importance of integrin signalling and Rho-dependent actomyosin contractility in multicellular tissue morphogenesis. This work also highlights the central role of active and isometric force in the functional integrity

of soft tissues such as the breast, where small changes in matrix stiffness or mechanical cues can profoundly alter cell behaviour.

Cancer: forcing transformation

Epithelial cancers are characterized by an altered tissue tensional homeostasis that reflects differences in rheology and increased cell-generated force in the transformed cells^{126–128}, increased compression force due to the solid state pressure exerted by the expanding tumour mass¹²⁹, matrix stiffening due to the desmoplastic response⁶, and increased interstitial pressure due a leaky vasculature and poor lymphatic drainage¹³⁰. For instance, transformed epithelial cells express vastly different intermediate filament profiles and cytoarchitecture to normal cells and consequently have an altered microrheology that could provide a distinct advantage to the cell during intravasation and extravasation of the vasculature, thereby facilitating cancer metastasis^{29,128,130}. Transformed cells also show compromised mechanoreciprocity such that they often exert abnormally high force in response to a compliant matrix and these increased cell-generated forces disrupt cell–cell junction integrity, compromise tissue polarity, promote anchorage-independent survival and enhance invasion (FIG. 4). It is also plausible that altered cellular force could account for the increased invadopodia⁶ observed in transformed, invasive cells¹³¹. Increased cell contractility probably reflects increased expression and activity of RhoGTPases and Rho-associated, coiled-coil-containing protein kinase 1 (ROCK1), as well as high levels of growth factor-induced Erk activity. The increased cell-generated forces exhibited by tumours enhance their growth, survival and invasion by promoting focal adhesion maturation and signalling through actomyosin contractility^{6,128,130,132–135}. The increased contractility of tumour cells and their associated stromal fibroblasts also induce tension-dependent matrix remodelling to promote the linear reorientation of collagen fibrils surrounding the invasive front of the tumour^{136,137}. Rapidly migrating transformed mammary epithelial cells have been observed on prominent linear bundles of collagen fibres adjacent to blood vessels^{138–140}.

The expanding tumour mass exerts compressive stress on the surrounding tissue extracellular matrix, vasculature, lymphatics and interstitial space. The solid stress induced by tumour expansion could also promote tumour progression. For example, tumours in soft tissues such as the pancreas typically show compromised laminin and type IV collagen basement membrane organization and thinning that, when combined with outward projecting compression force, facilitates tumour cell invasion into the parenchyma^{6,141} (FIG. 3). Tumour-associated compression stress can induce tumour angiogenesis by directly increasing expression of vascular endothelial growth factor A (VEGFA) or by indirectly blocking the existing vasculature surrounding the tumour mass to promote hypoxia and VEGFA secretion^{142,143}. In addition, compression can increase the interstitial pressure in the tumour to up to 10× that of normal tissue. This pressure induces the accumulation of fluid from leaky blood and lymphatic vessels^{144,145}. Compression force can also shrink the interstitial space surrounding the ductal structures, which increases the local concentration of growth factors and cytokines to facilitate autocrine and paracrine signalling and promote tumour growth¹⁴⁶. Tumour-associated changes in interstitial pressure and compressive stress also present real challenges for the treatment of solid tumours with chemotherapeutic drugs¹⁴⁷.

Breast cancer progression is accompanied by a desmoplastic response that includes inflammatory cell infiltration, angiogenesis, fibroblast transdifferentiation and changes in ECM composition, integrity and topology^{114,148,149}. The ECM remodelling observed in tumours includes increased deposition of fibronectin, tenascin, collagen types I, III and IV, and proteoglycans^{150–152}, substantial MMP-dependent cleavage and increased levels of LOX-dependent matrix crosslinking^{33,153}. In the normal breast, tightly controlled MMPs remodel the ECM to promote mammary gland growth or involution. In tumours, however, this

stringent control of MMP expression and function is lost¹⁵⁴. Overexpression of MMP3, MMP11, MMP12 and MMP13 have each been demonstrated in the tumour stroma, along with MMP2 in the transformed mammary epithelial cells¹⁵⁵. Moreover, aberrant MMP activity is not merely symptomatic of a transformed tissue but rather has a causative role, as illustrated by the observation that polymorphisms in the human MMP3 promoter that increase its expression are associated with an increased tumour incidence¹⁵⁶. Likewise, transgenic mice that overexpress MMP3 displayed marked desmoplasia and precocious branching of the mammary epithelium, and developed tumours with marked genetic abnormalities¹⁵⁷. However, tumour progression is also associated with substantial post-translational modifications of matrix proteins including altered deposition of proteoglycans and increased expression and activity of collagen crosslinking enzymes such as LOX and LOX^{158,159}. We showed that experimentally induced breast tumour progression in transgenic mice is accompanied by a significant increase in reversible and irreversible collagen crosslinking, increased expression of LOX and an incremental increase in tissue and ECM stiffness (K. Levental *et al.*, unpublished information). Inducing collagen crosslinking and stiffening either in three-dimensional collagen hydrogels or *in vivo* in a modified breast stroma promoted MEC transformation that was associated with increased mechanosignalling. Provocatively, inhibiting LOX-dependent collagen crosslinking tempered tissue desmoplasia, decreased tumour incidence, reduced tumour growth and reduced mechanotransduction in the mammary epithelium; thereby directly implicating changes in the properties of matrix materials in tumour evolution.

Tumour evolution is accompanied by dramatic changes in interstitial pressure and fluid flow. Fluid flow dynamics within soft tumour tissues has largely been ignored but is especially relevant to tissue development and tumour metastasis and for optimal treatment efficacy¹⁴⁵. For instance, fluid flow facilitates lymphatic clearance and induces cytokine differentials that promote cell motility and invasion through the creation of chemotactic C-C chemokine receptor 7 (CCR7) gradients that are highly important for cancer cell metastasis through the lymphatics¹⁶⁰. The increased interstitial pressure in an epithelial tumour mass, with fluids accumulating from leaky blood vessels and impaired lymphatic drainage, can greatly impede the delivery of tumour therapies¹³⁰. Clearly, tumour cells are exposed to a myriad of altered mechanical forces that could dramatically modify their behaviour. A better understanding of how these force cues regulate tumour progression and metastasis and affect cancer therapy could significantly aid the development of improved treatments¹⁶¹.

Breast density and age: a new perspective

Clinicians have long recognized that there is a connection between breast density and breast cancer risk^{6,162–164}. Increased mammographic density for instance, is associated with a four- to six-fold increase in the relative risk of developing breast cancer^{165,166}. Unfortunately, however, deciphering the functional relationship between mammographic density and breast transformation has proved quite challenging^{111,167}. For instance, although dense breasts have more collagen and increased cell density (reflected by a greater nuclear area), other factors such as altered levels of the tissue inhibitor of metalloprotease 3 (TIMP3) and insulin-like growth factor I (IGFI) are also associated with mammographic density and need to be considered^{165,168,169}. In fact, the composition of the ECM differs in women with dense breasts, such that the proteoglycans lumican and decorin are often disproportionately increased in women with mammographically dense breasts^{165,169}. Proteoglycan deposition often precedes fibrosis and may enhance tissue inflammation, raising the intriguing possibility that women with mammographically dense breasts could be more susceptible to chronic inflammation¹⁷⁰. Interestingly, proteoglycans such as lumican and biglycan not only bind growth factors and maintain tissue hydration but also contribute crucially to the mechanical integrity of the stroma, suggesting that in some instances mammographic density could reflect

a stiffer breast parenchyma^{36,171}. Given that matrix stiffness can modify cell and tissue behaviour by altering adhesion and growth factor receptor signalling and cytoskeletal dynamics to change cell shape and tissue organization, it seems reasonable to predict that the increased breast cancer risk associated with dense breasts could be attributed in some instances to an aberrant tensional homeostasis in these tissues. In this regard, sonoelastography, which measures the stiffness of a tissue in real time *in situ*, might offer a tractable auxillary screening strategy to diagnose high-risk women who are identified initially using imaging mammography¹⁷² (FIG. 5).

As women age, mammographic density decreases yet cancer incidence rises^{169,173}. Indeed, the post-menopausal breast has proportionately less collagen and more fatty tissue than the young breast, implying that older breast tissue must be softer¹⁶⁹. Consistently, in old skin and bone, collagen deposition decreases and MMP-dependent degradation increases, and old bone and skin are mechanically weaker than their younger tissue counterparts^{174,175}. How can we reconcile this seeming paradox between the increased cancer risk with age and the decreased mechanical strength of tissues? one explanation is that ageing is associated with a disproportionate increase in inappropriate post-translational modifications of ECM proteins, including increased collagen glycation and ultraviolet crosslinking, yielding old tissue matrices with less total collagen but a greater amount of disorganized collagen fibrils than young tissues. Consistently, although old skin has lower tensile properties (that is, is mechanically weaker) it is paradoxically stiffer (less elastic) and less functional than young skin^{176,177}. Wound healing in old skin is severely compromised, which could be attributed to altered mechanical properties of the extracellular collagens^{178,179}. Therefore, there is a positive association between age, matrix stiffening, aberrant matrix crosslinking and increased cancer incidence. Although the post-menopausal breast has less collagen, the collagen may be less mechanically elastic, stiffer, more disorganized and less functional, a possibility that now needs to be examined.

These findings underscore the need to understand the complex relationship between matrix remodelling and topology, and cell and tissue behaviour. Indeed, although hormone replacement therapy can increase breast density and tamoxifen treatment can reduce breast density, these mammographic changes do not always reflect modified cancer risk, emphasizing our need to develop additional metrics to understand the relationship between ECM remodelling and tissue phenotype^{23,180}. Such insight would be highly beneficial for clarifying those issues encountered with the recent clinical trials of MMP inhibitors in cancer treatment¹⁸¹.

Summary

Realizing that force is a crucial determinant of tissue development, cell differentiation and homeostasis leads us to conclude that the loss of the ability to sense, respond and adapt appropriately to force contributes to disease. Indeed, we and others showed that pathological changes in cells and in the architecture, topology and material properties of their matrix microenvironments constitutes a positive feedback loop that propels carcinogenesis and other diseases. However, many questions still need to be resolved. Such issues include how the unique material properties of specific differentiated tissues are established and maintained, how cells coordinate their function and adaptation to external cues with their microenvironments, and how physical signals might interface with and modulate the activity of biochemical signalling pathways. Addressing these questions is particularly important if we are to understand lethal processes such as tumour metastasis, which clearly is profoundly influenced by the primary tissue microenvironment. Metastasis is also acutely regulated by the inherent cellular rheology and the forces that the cells experience during their metastatic spread, and is chronically regulated by the material properties of their targeted distal metastatic

niche^{29,182}. It may be that this niche is defined by proteins such as LOX or TGF β , which have the capacity to modify tumour cell adhesion and the material properties of ECM in tissues targeted by these cells, respectively^{183,184}. In this regard, recent work suggests that tumour cells select their metastatic microenvironments in part through compliance matching but also by pre-conditioning their metastatic niche. This raises a number of intriguing questions, including defining the part that mechanical force might play in modulating the function of tumour stem cells, why specific tumour types characteristically metastasize to distinct tissues, and whether tumour cells might be mechanically pre-conditioning their metastatic sites. Clearly, addressing such outstanding issues falls outside the realm of traditional cell biology approaches and instead requires the cooperative effort of biologists, materials scientists, physicists and engineers. Indeed, this exciting force ‘frontier’ is fertile territory for scientific exploration of development and cancer biology that will undoubtedly yield new insights into cancer evolution and identify novel anticancer therapeutic targets.

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Glossary

Rheology

The study of the deformation and flow of matter.

Viscoelasticity

Soft biological tissues can be described as viscoelastic materials. A viscous fluid resists shear flow and strain linearly with time under stress. An elastic solid undergoes deformation under stress and rapidly returns to its original state. Viscoelastic biological materials exhibit characteristics of both a viscous fluid and an elastic solid.

Endoproteinase

An enzyme that proteolytically cleaves peptides at internal amino acids.

Durotactic

Directed movement of cells up or down the stiffness gradient of a biomaterial.

Desmoplastic stroma

Stromal tissue responds to tumour cells with a characteristic desmoplasia resulting from fibroblast recruitment, collagen deposition and angiogenesis.

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Biographies

Darci T. Butcher received her Honours bachelor degree in Genetics and her Ph.D. from the University of Western Ontario, Canada. Her postdoctoral studies in Valerie Weaver’s laboratory explored the role of cell and matrix tension in epigenetic regulation of mammary epithelial immortalization and tumour progression.

Tamara Alliston received her B.A. at Trinity University in San Antonio, Texas, USA and her Ph.D. at Baylor College of Medicine, Texas, followed by postdoctoral research at the University of California at San Francisco (UCSF). She is currently an assistant professor in the Department of Orthopaedic Surgery at UCSF and is affiliated with the Institute of Regeneration Medicine, the Departments of Bioengineering and Otolaryngology, and graduate programmes in Biomedical Sciences, Bioengineering, and Oral and Craniofacial Sciences. Her research focuses on mechanisms by which growth factors coordinately regulate cell differentiation and extracellular matrix material properties in skeletal development and disease.

Valerie M. Weaver received her Bachelor in Chemistry and Biochemistry from the University of Waterloo, Ontario, Canada and her Honours Bachelor summa cum laude and Ph.D. in Biochemistry from the University of Ottawa. After completing postdoctoral training with M. J. Bissell at the Lawrence Berkeley National Laboratory in Berkeley, California, USA she joined the faculty of Pathology at the University of Pennsylvania, Philadelphia, Pennsylvania as an Assistant Professor and was a member of the Institute for Medicine and Engineering. She relocated to UCSF where she is Associate Professor and Director of the Department of Surgery's Center for Bioengineering and Tissue Regeneration. She has cross appointments in the Departments of Anatomy and Bioengineering and Therapeutics at UCSF and is Adjunct Associate Professor in the Department of Bioengineering at the University of Pennsylvania. Using an integrated, interdisciplinary approach her group studies the role of mechanical force and stromal–epithelial interactions in tissue development, tissue-specific differentiation and breast cancer.

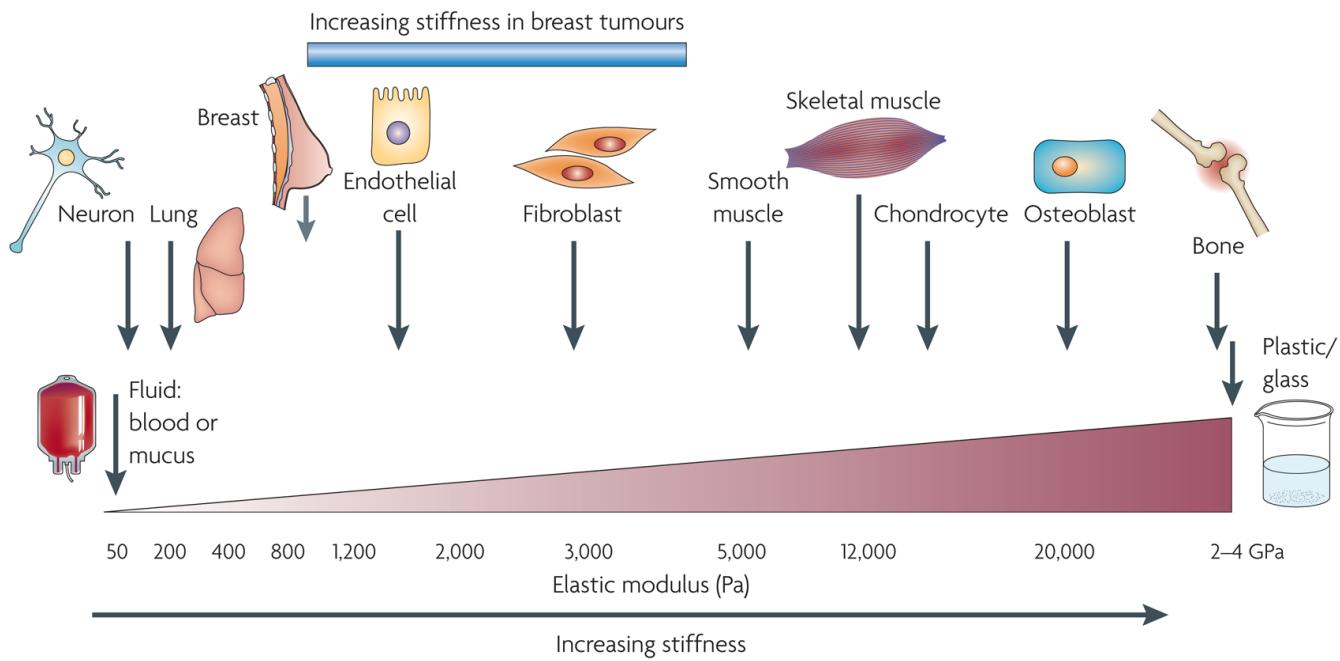


Figure 1. Cells are tuned to the materials properties of their matrix

All cells, including those in traditionally mechanically static tissues, such as the breast or the brain, are exposed to isometric force or tension that is generated locally at the nanoscale level by cell–cell or cell–extracellular matrix interactions and that influences cell function through actomyosin contractility and actin dynamics. Moreover, each cell type is specifically tuned to the specific tissue in which it resides. The brain, for instance, is infinitely softer than bone tissue. Consequently, neural cell growth, survival and differentiation are favoured by a highly compliant matrix. By contrast, osteoblast differentiation and survival occurs optimally on stiffer extracellular matrices with material properties more similar to newly formed bone. Normal mammary epithelial cell growth, survival, differentiation and morphogenesis are optimally supported by interaction with a soft matrix. Following transformation, however, breast tissue becomes progressively stiffer and tumour cells become significantly more contractile and hyper-responsive to matrix compliance cues. Normalizing the tensional homeostasis of tumour cells, however, can revert them towards a non-malignant phenotype⁶, thereby illustrating the functional link between matrix materials properties, cellular tension and normal tissue behaviour. Importantly, however, although breast tumours are much stiffer than the normal breast, the materials properties of a breast tumour remain significantly softer than those of muscle or bone, emphasizing the critical association between tissue phenotype and matrix rigidity.

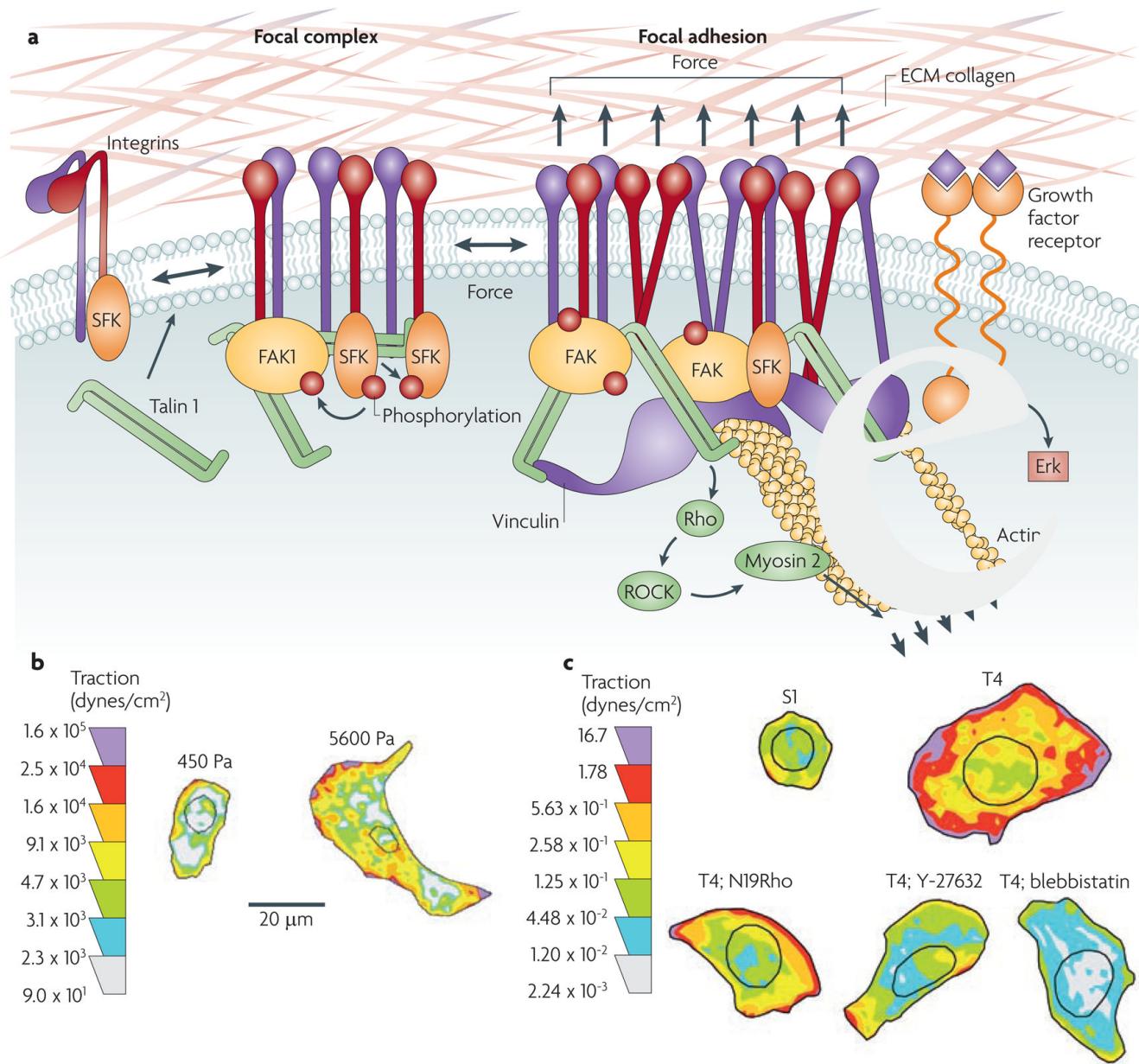


Figure 2. Mechanotransduction and focal adhesion maturation

a | The majority of integrins exist at the plasma membrane in a resting, inactive state in which they can be activated by inside-out or outside-in cues. With regard to outside-in activation, when cells encounter a mechanically rigid matrix or are exposed to an exogenous force integrins become activated, which favours integrin oligomerization or clustering, talin 1 and p130Cas protein unfolding, vinculin–talin association, and Src and focal adhesion kinase (FAK) stimulation of RhoGTPase-dependent actomyosin contractility and actin remodelling. Focal adhesions mature with the recruitment of a repertoire of adhesion plaque proteins, including α -actinin to facilitate actin association, and adaptor proteins such as paxillin, which foster interactions between multiple signalling complexes to promote growth, migration and differentiation. **b** | Normal cells tune their contractility in response to matrix stiffness cues, but tumours exhibit altered tensional homeostasis. Cells exert actomyosin contractility and

cytoskeleton-dependent force in response to matrix stiffness cues. These forces can be measured using traction force microscopy. Thus, non-malignant human mammary epithelial cells spread more and exert more force on a stiff matrix than on a soft matrix. **c** | By comparison, breast tumour cells (T4) are highly contractile and spread considerably more than their non-malignant counterparts (S1) in response to the same compliant matrix. Importantly, inhibiting RhoGTPase signalling in tumour cells, by expressing a dominant-negative N19Rho or treating tumours with an inhibitor of Rho-associated, coiled-coil-containing protein kinase (ROCK; Y-27632) or myosin 2 (blebbistatin), reduces tumour cell contractility and spreading to levels exhibited by non-malignant breast epithelial cells. These data illustrate the importance of Rho signalling and actomyosin contractility in cell force generation and show how transformation alters cell force sensing. The traction map is shown in pseudocolour indicating regions of low (grey) and high (purple) forces in dynes per cm^2 . ECM, extracellular matrix; SFK, Src family kinase. Reproduced, with permission, from REF. ⁶ © (2005) Elsevier Inc.

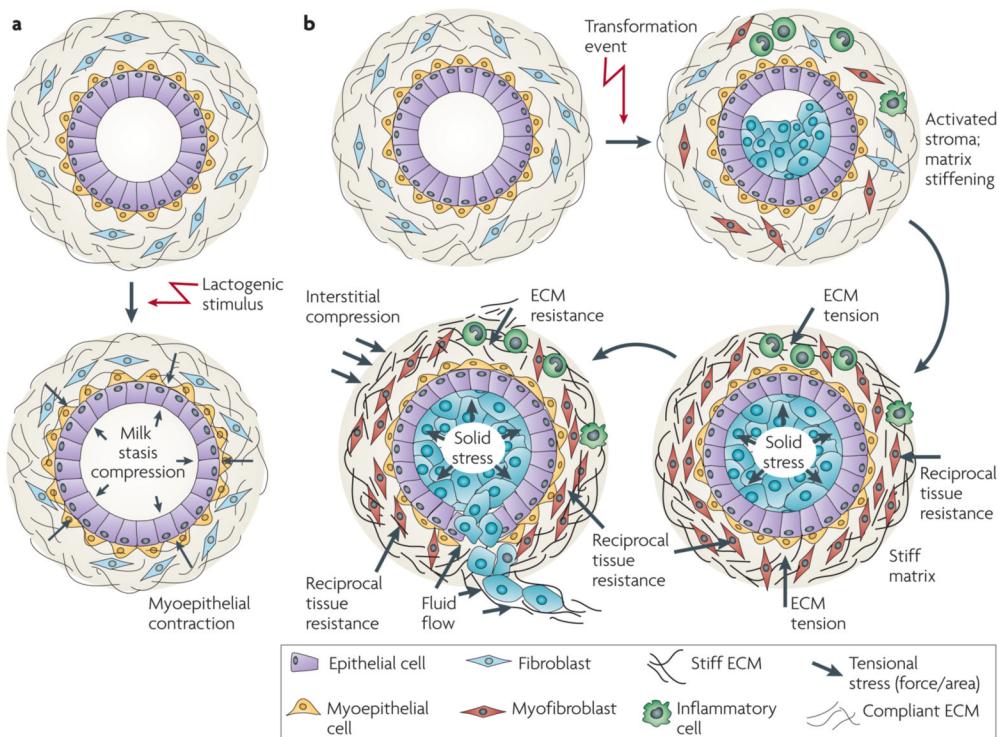


Figure 3. The normal mammary gland as a mechanically active tissue

a | The developing breast is subjected to a number of forces that facilitate its normal function. During lactation, for instance, the normal breast experiences compressive stress on the luminal epithelial cells and the basement membrane owing to the accumulation of milk and alveolar distension. Upon sucking and oxytocin stimulation, epithelial cells encounter inward tensile stress as the myoepithelium contracts to force the milk out of the alveolar sacs. In the absence of this stimulus, milk will accumulate within the acinus and eventually exert an outward projecting compressive force on the surrounding epithelium. This compressive force is countered by a compensatory inward projecting resistance force and the combination of these two forces eventually compromises the integrity of the tight junctions between alveolar cells. Chronic exposure to these forces and perturbed tissue integrin sensitizes the gland to apoptotic cues so that the gland undergoes involution accompanied by extensive remodelling of the epithelium and the cellular and extracellular components of the stroma. **b** | Transformation (blue cells) resulting from the accumulation of genetic and epigenetic alterations in the epithelium along with an altered stromal matrix leads to unchecked proliferation and enhanced survival of luminal epithelial cells within the ductal tree, which compromises normal ductal architecture. With prolonged growth and abnormal survival, the abnormal pre-neoplastic luminal mammary epithelial cells eventually expand to fill the breast ducts. The expanding luminal epithelial mass exerts outward projecting compression forces of increasing magnitude on the basement membrane and adjacent myoepithelium. These forces are countered by an inward projecting resistance force. Importantly, the pre-neoplastic lesion secretes a plethora of soluble factors that stimulate immune cell infiltration and activation of resident fibroblasts to induce a desmoplastic response in the breast stroma. The desmoplastic stroma, which is characterized by dramatic changes in the composition, post-translational modifications and topology of the extracellular matrix (ECM), stiffens over time. This rigid parenchyma exerts a progressively greater inward projecting resistance force on the expanding pre-neoplastic duct. Over time, the number of myoepithelial cells surrounding the pre-neoplastic mass decreases and the basement membrane thins, probably owing to increased matrix metalloproteinase

(MMP) activity, decreased protein deposition and compromised assembly (adapted from REF. 128). In parallel, there is a build-up of interstitial fluid pressure contributed by a leaky vasculature and compromised lymphatic drainage. In response to their genetic modifications and the altered materials properties of the matrix, the pre-neoplastic luminal epithelial cells exhibit modified tensional homeostasis and respond to the combination of forces and stromal cues to invade the breast parenchyma. Some resident fibroblasts transdifferentiate into myofibroblasts and facilitate tumour migration and invasion by promoting the assembly of linearized collagen fibrils surrounding the distended pre-neoplastic epithelial ducts.

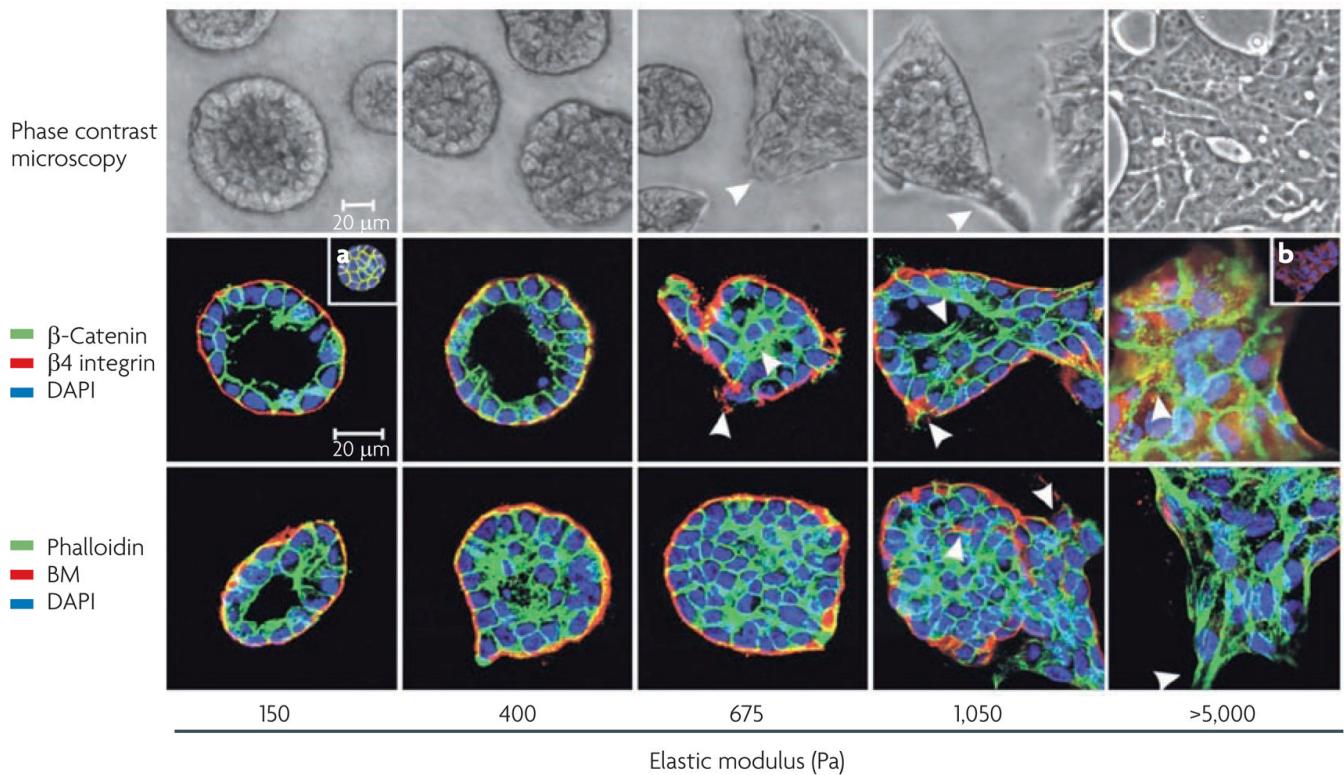


Figure 4. Matrix stiffness modulates cellular morphology and epidermal growth factor (EGF)-dependent growth

Phase contrast microscopy and confocal immunofluorescence images of non-malignant immortalized human mammary epithelial cell (HMEC; MCF10A) colonies interacting with a three-dimensional reconstituted basement membrane (BM)-laminated polyacrylamide gel of increasing stiffness (150–5,000 Pa) showing colony morphogenesis after 20 days of culture. On compliant gels with materials properties similar to that measured in the normal murine mammary gland (150 Pa) non-malignant MECs proliferate for 6–12 days to eventually form growth-arrested, polarized acini analogous to the terminal ductal lobular units observed at the end buds of the differentiated breast. These structures have intact adherens junctions and insoluble cell–cell localized β -catenin before (main images) and after (inset a) Triton extraction, and polarity, as shown by the basal localization of $\alpha 6$ $\beta 4$ integrin, the apical–lateral localization of cortical actin (Phalloidin), and the assembly of an endogenous laminin 5 basement membrane. Incremental stiffening of the basement membrane gel progressively compromises tissue morphogenesis and alters EGF-dependent growth of these cells. Thus, colony size progressively increases with matrix stiffening, lumen formation is compromised, cell–cell junctions are disrupted, as revealed by loss of cell–cell-associated β -catenin (inset b), and tissue polarity is inhibited, as indicated by disorganized $\alpha 6$ $\beta 4$ integrin localization and loss of the endogenous laminin 5 basement membrane. Interestingly, actin stress fibres were not observed in the structures until the stiffness of the matrix reached 5,000 Pa, as has been observed in murine breast tumours *in vivo*⁶. The arrows indicate loss of the endogenous basement membrane and disruption of basal polarity. Reproduced, with permission, from REF. 6 © (2005) Elsevier Inc.

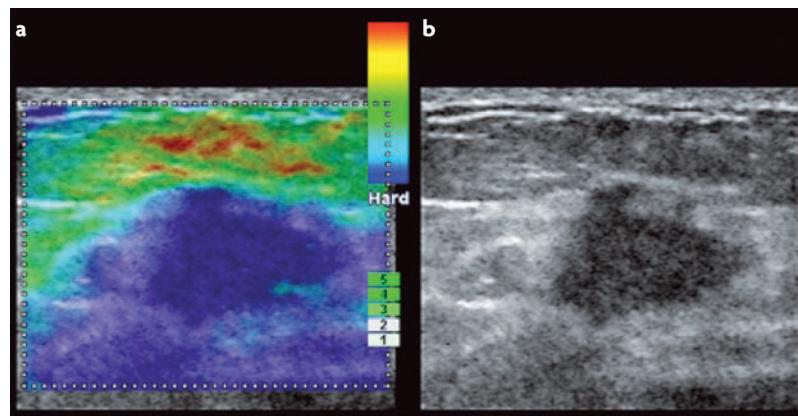


Figure 5. Imaging elastography of a breast tumour

Tissue imaging elastography is a spatial 'visual' qualitative measurement of the stiffness of a tissue that is generated by extrapolating tissue viscoelastic characteristics from ultrasound wave reflection in real-time. Photographs of sonoelastography images compare an elastogram image (a) with a B mode ultrasound scan (b) of a breast tumour¹⁷⁰. Ultrasound imaging elastography, as shown here, is an *in situ* mechanical imaging method that could improve the sensitivity and the specificity of breast cancer detection and may be a useful tool to advance our understanding of the link between mammographic density and the matrix materials properties of the breast. Image courtesy of A. Thomas & T. Fischer, Charité, Berlin, Germany.